Bioassay of Senna Leaves and of the Fluidextract of Senna, U. S. P. XI

*By E. Geiger, M.D.**

The widespread use of senna leaves is indicated by the official statistics which show that, in 1934, 1,303,386 pounds were imported by the United States. In spite of the generally satisfactory therapeutic action, there are, frequently, complaints in connection with this drug. Its disadvantages are: the undesirable by-effects, such as considerable gripes and a disagreeable taste which makes the infusion unpalatable for many individuals.

Therefore, the pharmaceutical industry has endeavored to separate the therapeutically active components from the ballast substances and to make them into a palatable preparation, so that this laxative can be taken, even by sensitive people. This has led to the production of the official Fluidextract, Syrup, etc., and proprietary preparations. However, the results have been far from satisfactory, so that the home-made infusion from the leaves is still preferred to the ready-made preparations.

During the past several years, we have tried to isolate the active substances from senna leaves, but we were handicapped by the lack of a means to determine the potency of the isolated products. We were restricted to self-experimentation and to results reported by healthy volunteers, who took the samples. On the whole, these reports were conflicting and often misleading. Hence, we tried to develop an objective method for quantitative determination of of the activity of senna preparations. Our first experiments, performed on isolated parts of the digestive tract of different animals, were unsuccessful, so we turned our attention to living animals and used mice for their easy access and handling.

Earlier authors (1) have experimented on mice and their results have been reviewed by Munch, (2) who concluded that "the assay of cathartics upon mice appears promising but further study is necessary to standardize the technic."

* From the Department of Physiology and Pharcology of The Chicago Medical School.

EXPERIMENTAL

White mice, weighing 20 to 24 Gm. each, were used; but only *males* as the females usually are more expensive. It appears to be unnecessary to use animals of the same breed, as we found that mice raised in Budapest, Buffalo, Chicago or Milwaukee gave almost the same results.

The most important factor for satisfactory results is the temperature of the animal room. Mice are very sensitive to low temperatures, and usually the first results of exposure to cold is diarrhea. For this reason, the temperature of their room must be kept above 20° C.

As the consistency of the stool depends on the kind of *food* fed to the animals, such foods should be selected as will result in solid and dry stools. At the beginning of the experiments, we fed the mice oats with satisfactory results, except that this exclusive diet reduced the appetite and consequently they lost weight. We now feed with Dickinson's "Dog-food" which contains all the necessary ingredients. The stools have the right consistency and the animals like this food so that their condition is very good, even during prolonged experiments.

We thought that, before the experiment, the food should be withdrawn, but found that it was not satisfactory to starve the mice, even for 5 or 6 hours, as their digestive tracts then had varying contents; if the starving continued longer than 5 or 6 hours, the mice were weakened, and thus the uniformity of results was disturbed by the reduced resistance of some animals. Now, therefore, we do not starve the animals at all, but let them have their food before and during the experiment. We only discharge their water before the experiment.

Method.—Move the mice from their cages to individual glass beakers of 1-liter capacity. Put two layers of filter paper at the bottom of the beaker, and, in order to prevent the mice from lifting and gnawing the paper, cover it with a round wire mesh, the openings of which are 0.5 x 0.5 cm. After half an hour, check the feces of each animal and discharge those mice from the experiment which do not have a hard stool. However, soft stools are very rarely the case and are, usually, a sign of too low a room temperature. Then introduce 0.5 cc. of the senna extract or infusion into the stomach of those animals which are suited for the experiment. This can be done with a tuberculin syringe with an attached blunted needle, 7 cm. long and No. 16 gage. It is unnecessary to tie the mice down as, after some practice, the needle can be easily inserted into the stomach, holding the mouse in the hand. After introducing the solution into the stomach, put the animal back into its beaker, give one cube of the "Dog-food" and keep under control for twelve hours. We consider the result as "positive" if, within this time, the stool

becomes pasty and marks the filter paper brown by imbibition.

We ascertained in preliminary experiments, that 0.5 cc. of water, or 0.5 cc. of 0.2% carbonate solution, introduced into the animal's stomach, did not result in diarrhea, but the stool remained solid and dry.

Usually we prepared fresh 5 per cent infusions of the senna leaves,[1] adding 0.4 Gm. of anhydrous sodium carbonate to each 5 Gm. of leaves to prevent hydrolysis of the glycosides.

Table I shows that 0.5 cc. of a 5% infusion, diluted 1:4, causes a cathartic action in approximately 10% of the animals. The dilution 1:3 acted on 39% and the dilution 1:2 on 86% of them. Table I shows also that the same group of animals react in the same way, in regard to the percentage of positive results, on different days and different groups of animals give approximately the same quantitative results. Table I shows finally that, after a four days' interval, the animals give again satisfactory results.

The following experiments (see Table II) show that the same group of animals, which were kept under observation over a period of three months, give practically the same results in weekly performed experiments. This proves that the same animals can be used for these experiments without their becoming over-sensitive or addicted to the drug, providing that they are given the necessary rest between each experiment.

Table I.—Cathartic Action of Senna Infusion

Date	Group of Mice	Dilution of 5% Infusion	Number of Mice	No. of Positive Results	Percentage of Positive Results
V. 8	*A*	1:2	12	12	100%
V. 29	*A*	1:2	12	10	83%
VI. 28	*A*	1:2	12	11	91%
V. 22	*D*	1:2	12	9	75%
V. 18	*E*	1:2	8	6	75%
V. 29	*E*	1:2	8	8	100%
VI. 14	*E*	1:2	8	6	75%
		Total	72	62	Ave. 86%
V. 18	*A*	1:3	12	6	50%
V. 24	*B*	1:3	10	3	30%
VI. 2	*B*	1:3	10	4	40%
VI. 12	*B*	1:3	10	3	30%
V. 28	*D*	1:3	12	4	33%
VI. 3	*E*	1:3	8	3	37%
V. 19	*F*	1:3	12	5	42%
VI. 5	*F*	1:3	12	6	50%
		Total	86	34	Ave. 39%
VI. 10	*A*	1:4	12	2	16%
V. 15	*B*	1:4	10	1	10%
VI. 5	*D*	1:4	12	1	8%
VI. 18	*E*	1:4	8	0	0%
V. 23	*F*	1:4	12	2	16%
VI. 20	*F*	1:4	12	1	8%
		Total	66	7	Ave. 10%

[1] We obtained senna leaves through the courtesy of Dr. Thomas Lewis from S. B. Penick & Co. Unless otherwise stipulated, we used "Alexandria Half Leaves," delivered by this company.

Table II.—Repeated Experiments on the Same Group of Mice

Date	Group of Mice	Dilution of 5% Infusion	Number of Mice	No. of Positive Results	Percentage of Positive Results
X. 9	*C*	1:3	20	8	40%
X. 18	*C*	1:3	20	9	45%
X. 26	*C*	1:3	20	8	40%
XI. 2	*C*	1:3	19	7	37%
XI. 14	*C*	1:3	19	6	31%
XI. 23	*C*	1:3	16	7	43%
XII. 4	*C*	1:3	16	6	37%
XII. 11	*C*	1:3	15	7	46%
XII. 18	*C*	1:3	15	8	53%
				Ave.	43%

The amount of experimental data appears to be too small as yet to enable an evaluation of the results by means of statistical methods. We are engaged in further experiments in order to determine the limit of error; we are also trying to obtain a curve which will enable us to establish the potency of the preparation by interpolation. Tables I and II show decisively, however, that the described method is sufficiently established for practical application.

Practical Application.—To determine the potency of an unknown senna or its preparation we used, as a standard, the 5 per cent infusion made from "Alexandria Half Senna" leaves, diluted in water 1:3. The potency of the test solution was compared with this standard solution. The procedure is as follows:

Form three groups of 7 to 12 animals each; feed these mice for six days as previously referred to. Give group *A*, 0.5 cc. of the standard solution; group *B*, 0.5 cc. of the 1:2 diluted test solution; and group *C*, 0.5 cc. of the 1:4 diluted test solution. Four days later, give group *B* or *C* the standard solution, and the two other groups the test solution diluted 2, 2.5, 3 or 4 times, according to the result of the preceding experiment, in order to find a dilution of the test solution which equals in potency the standard solution.

As an example, we describe here the following experiment to establish the potency of senna leaves, purchased in a drug store (see Table III).

Table III shows that the infusion prepared from the purchased senna leaves, diluted 1:2.5, is about strong as the standard solution, diluted 1:3. This means that the tested leaves have approximately 80% of the potency of the standard leaves.

In another experiment we purposely mixed 5 Gm. of standard leaves with 5 Gm. of leaves already extracted and dried (*i. e.*, inactive leaves), and prepared an infusion from this mixture. The results show that the test solution, 1:3 dilution, had no effect; the test solution, diluted 1:2, gave 20% and 15% positive results, respectively. The undiluted infusion gave 80% positive results. These results go to show that the mixture we used had only approximately half of their original activity.

Table III.—Bioassay of Senna Leaves

	Group of Mice	5% Infusion	Number of Mice	No. of Positive Results	Percentage of Positive Results
First test	*A*	Standard dilution 1:3	12	6	50%
	B	Test dilution 1:2	12	8	66%
	C	Test dilution 1:4	12	..	0%
Cross test	*A*	Test dilution 1:2.5	12	5	41%
	B	Standard dilution 1:3	12	4	33%
	C	Test dilution 1:3	12	3	25%

Examination of Senna Preparations.—The previous experiments having shown that the mouse test is suitable for the bioassay of senna leaves, experiments were undertaken to prove that these methods were suitable also for the extractive preparations of senna.

Fluidextract of Senna, U. S. P. XI, is supposed to be prepared in such a way that 1 cc. of the Fluidextract is equal to one Gm. of the leaves. As shown above the extract of 0.008 Gm. of leaves in 0.5 cc. of water is the effective dose for 40% of the mice of 20 Gm. weight. Therefore, we had to assume that 0.5 cc. of the 60-times diluted Fluidextract would be the proper cathartic dose. We prepared the 1:60, and also a 1:30 dilution of the Fluidextract, and administered 0.5 cc. of each to the test animals. However, both dilutions were uneffective on the mice.

The Fluidextract contains, according to the label, 25% of ethyl alcohol, so it was possible that the alcohol inhibited the cathartic action of the extract. To examine this assumption, we prepared a 5% infusion as described above and added to it different amounts of ethanol in such proportions that the mice received 2.5, 2.0, 1.5, 10 and 0.5% ethanol in 0.5 cc. of the infusion. 2.5% being the alcohol content of a Fluidextract diluted 1:10.

These experiments revealed that the added alcohol in higher concentrations caused a transitory narcosis, but did not inhibit the cathartic action.

Table IV.—Cathartic Action of Infusions Containing Ethanol

Dilution of the 5% Infusion	Alcohol-Content in %	Positive Reaction in %	Narcosis
1:3	2.5	50	For 10 min.
1:3	2.0	40	Mice quiet
1:3	1.5	40	Mice quiet
1:3	1.0	30	.....
1:3	0.5	50	.....

As the ethanol-content of the Fluidextract does not disturb the biological test, we tried to determine the effective concentration of the Fluidextract in the following experiments. We gave the animals 0.5 cc. of the Fluidextract diluted with water in the proportion of 1:20, 1:15 and 1:10, and found the 1:15 dilution as active as the 5% standard infusion diluted 1:3. As the 0.5 cc. of diluted (1:3) standard infusion is equivalent to 0.008 Gm. of leaves, 1 cc. of the Fluidextract is equivalent to 0.008 Gm. × 2 × 15 or 0.24 Gm. of leaves; *i. e.*, about 25% of the required official activity.

Disconcerted by these results, we presumed at first that the fluidextract used in the previous experiments might have been of a weak batch, or reduced in its activity by improper storage. Therefore we investigated different fluidextracts, made by several manufacturing houses and found that these fluidextracts contained only 15 to 25% of the supposed activity.

We see from Table V that the fluidextracts examined are practically equal in their activity, with the exception of *C*, but far below their expected potency. The cathartic action of 1 cc. corresponds only with 0.2 to 0.3 Gm. of leaves instead of 1 Gm. This result was confirmed by many statements from individuals saying that, in order to obtain a cathartic action in man, it is necessary to take 15 to 20 cc. of the Fluidextract, although we know that, in form of an infusion, 2 Gm. of leaves are sufficient.

Table V.—Bioassay of Fluidextracts, U. S. P. XI

Fluid-extract	Dilution	Number of Mice	Positive Reaction in	Percentage of Positive Results
A	1:20	10	0	0
	1:15	12	4	33
	1:10	12	10	85
B	1:20	20	0	0
	1:15	20	4	20
	1:10	20	16	80
	1:5	20	20	100
C	1:20	10	0	0
	1:15	20	0	0
	1:10	20	2	10
	1:5	20	6	33
D	1:20	20	0	0
	1:15	20	8	40
	1:10	20	18	90
E	1:20	12	1	8
	1:15	12	4	33
	1:10	12	11	92
F	1:20	20	1	5
	1:15	20	4	20
	1:10	20	17	85

Founded on these experiments we assumed that the method used for preparing the fluidextract is not satisfactory.

At the beginning of these experiments, we noted that the fluidextracts were usually acidic in reaction. In experiments with the infusion we have seen, however, and in confirmation of Straub's statement, (3) that alkaline extracts are more potent, as the active glycosides are hydrolyzed at low p_H. To check this influence of the p_H Prof. E. N. Gathercoal was kind enough to furnish us with a fluidextract of a p_H 7.11, prepared by adding Na_2CO_3 to the menstruum thus neutralizing the organic acids of the leaves.

This extract was stronger than those previously tested: 1 cc. corresponds with 0.30 to 0.40 Gm. of the leaves.

Table VI.—Activity of Alkaline Fluidextract

Dilution of Fluidextract	Number of Mice	No. of Positive Results	Percentage of Positive Results
1:10	10	10	100%
1:20	20	9	45%
1:25	20	4	20%
1:30	20	0	0%

This experiment shows that it is advisable to use an alkaline menstruum for the extraction, in order to obtain a fluidextract with a p_H higher than 7.

To ascertain if the relatively low activity of the different fluidextracts was due to imperfect extraction of the leaves, we examined the marc which remained after preparing the above tested alkaline fluidextract. The infusion made from the dried marc was practically without any cathartic effect. Thus the active principles were not left in the marc, but must have been lost during the process.

The repeated examination of the alkaline fluidextract showed a progressive deterioration of activity, and thus gave us a clue to the cause of its weak potency. This deterioration was accompanied by a progressive accumulation of the sediment at the bottom of the flask. We separated this sediment by centrifugation, dissolved it in water and found it very active in the mouse test.

Experiment 1.—Fifty grams of Fluidextract *B* (see Table V) were centrifuged at 2000 r. p. m. for 10 minutes. After decanting the fluid, 3.5 Gm. of precipitate were collected and dissolved in 30 Gm. of water. Each mouse received 0.5 cc.

Table VII.—Cathartic Action of the Sediment Separated from a Fluidextract

Dilution	Number of Mice	No. of Positive Results	Percentage of Positive Results
1:5	10	10	100%
1:10	10	8	80%
1:15	10	3	30%

The effective dose of the 1:1 dilution corresponds to 0.008 Gm. of leaves; therefore the 30 cc. of the undiluted solution of precipitate is equivalent to 7.2 Gm. of leaves. The 50 cc. of fluidextract are equivalent to 12 Gm. of leaves (see Table V), hence the total activity of fluidextract *B* existed to the extent of about 60% in the precipitate and 40% in the decanted fluid.

Experiment 2.—Forty grams of Fluidextract *F* yielded 3.1 Gm. of precipitate; dissolved in 30 cc. of water, 0.5 cc. of the solution (1:10) corresponds to 0.008 Gm. of the leaves; 3.1 Gm. of the precipitate equal 4.8 Gm. of the leaves.

According to Table V, 40 Gm. of the fluidextract equal 9.6 Gm. of the leaves; therefore, 66% of total activity is in solution and 33 per cent is in the sediment.

Further experiments revealed that the precipitation was caused by the ethanol contained in the fluidextract; in fluidextracts from which the alcohol was eliminated *in vacuo*, no precipitation occurred. We found, further, that when we dissolved the sediment no precipitate was formed, but upon adding ethanol up to 25% a sedimentation again took place. From these experiments we conclude that the loss of activity is caused by the sedimentation of active material through alcohol.

It also appears interesting that there is a qualitative difference between the action of the fluidextract or the infusum and that of the sediment; with the former the cathartic action sets in after a latency of 4 to 6 hours, but the precipitate acts after a shorter time—1 to 2 hours.

We believe that some griping substances, like emodin, which are soluble in 25% ethanol, are responsible for delaying the purgative effect. This assumption is confirmed by the fact that the watery solution of the sediment does not show the Bornträger reaction, but the fluidextract shows it strongly.

In order to give a further proof, we extracted 10 Gm. of senna leaves for 12 hours with 100 cc. of ethanol and prepared an infusion from the extracted and dried leaves. The bioassay of this infusion showed that the treatment of the leaves with ethanol reduces the activity of the leaves 60 to 70% of the original potency. As the ethanol extract did not show activity, it was to be assumed that the active material has been partly destroyed by the alcohol treatment (alcoholysis of the glycosides?).

After some preliminary experiments we found the best way for the preparative extraction to be as follows: Treat the leaves with 10 times the amount of a mixture of absolute ethanol and methanol (70:30), with the addition of one cube of ammonium carbonate. This cube dissolves only partly and is used for the neutralization of organic acids of the leaves. Shake repeatedly, during 12 hours, decant the alcohol and remove the remainder of the ammonium carbonate cube. Prepare an infusion from the dried leaves; this is fully active in spite of the previous treatment of the leaves with alcohol.

Table VIII.—Assay of Alcohol-Treated Leaves

Dilution	No. of Animals	No. of Positive Results	Positive in %
1:4	20	2	10
1:3	20	8	40
1:2	20	20	100

The laxative effect appeared very rapidly in these experiments, *i. e.*, after a latency of 1.5 to 2 hours. To ascertain that the substancess eliminated by the preparatory alcoholic extraction usually delay the cathartic effect, we evaporated the alcoholic extract of 5 Gm. of leaves *in vacuo* and dissolved the remainder in the infusion, corresponding with 5 Gm. of alcohol-extracted leaves. Giving this infusion to the mice, the laxative effect appeared only after 3 to 4 hours.

These experiments have shown that by alcoholic extraction some substances have been actually eliminated from the leaves, these being substances which delay the purgative action of senna.

The preparatory extraction with alcohol had also the advantage that some of the unpalatable and bitter substances are also removed so that the watery extract has a pleasant taste.

On the basis of these experiences, we tried to prepare a highly active fluidextract through concentration of an infusion, made from alcohol-treated leaves. The infusion was evaporated to one-fifth of its volume *in vacuo*, so that 1 cc. corresponded to 0.5 Gm. of leaves. It resulted in a fluidextract of syrupy consistency.

The results of the bioassay of this fluidextract are shown in Table IX.

Table IX.—Assay of Fluidextracts Prepared by a Method Discussed in this Paper

Extract	Dilution	Animals Used	No. of Positive Results	Positive in %
M	1:20	12	11	91
	1:30	12	3	25
	1:40	12	1	10
N	1:20	10	8	80
	1:30	10	3	30
	1:40	10	2	20
O	1:20	10	9	90
	1:30	10	4	40
	1:40	10	0	0

As 0.5 cc. of the 1:30 diluted extract equals 0.008 Gm. of leaves, 0.5 cc. of the undiluted extract is equal to 0.24 Gm. of leaves and 1 cc. equals 0.48 Gm. of leaves.

The data of Table IX show that the cathartic activity is also practically equal to the potency of 0.5 Gm. of leaves. This means that the extract prepared by the described method contains all of the cathartic activity of the original material, so that no loss has occurred during the process.

With the help of the bioassay, we have succeeded in showing that the U. S. P. Fluidextract of Senna is not satisfactory in activity and that the factors responsible for this low activity can easily be avoided.

SUMMARY

1. A simple method for the bioassay of senna leaves and senna preparations is described, based on the cathartic action of the senna principles on mice. The amount of experimental data obtained till now is not sufficient for the statistical evaluation of the method; it has been shown, however, that the discussed method is quite suitable for practical purposes.

2. With the help of this method, it has been shown that the low p_H and the alcoholic content of the U. S. P. Fluidextract of Senna diminish the cathartic activity of this preparation.

The author is indebted to Professor E. N. Gathercoal of the University of Illinois School of Pharmacy for his helpful interest in the present experiments.

REFERENCES

(1) Führer, H., *Arch. exp. Path. Pharmakol.*, 105 (1925), 251; Loewe, S., and Faure, G., *Ibid.*, 107 (1925), 170

(2) Munch C. J., "Bioassays: A Quantitative Handbook of Pharmacology," page 773. William and Wilkins Co., Baltimore, 1931.

(3) Straub, W., and Gebhardt, H., *Arch. exp. Path. Pharmakol.*, 181 (1936), 400.

Absorption and Toxicity of Sodium and Potassium Thiocyanates*

By Robert C. Anderson and K. K. Chen†

Since the observation of Pauli (1) in 1903 that sodium thiocyanate (synonymous with sulfocyanate or rhodanate) lowered the blood pressure in hypertension, numerous clinical reports have appeared in medical literature. Table I summarizes the articles of American and Canadian origin. Briefly speaking, sodium or potassium thiocyanate when properly used reduces both systolic and diastolic pressures in patients with hyperpiesis, and causes subjective improvements. Toxic symptoms of various forms, however, may occur frequently. Thus extensive cutaneous lesions have been repeatedly recorded by Logefeil (7), Weis and Ruedemann (25), Ayman (26), Tyrrell (27), Baker and Brunsting (28), Green and Snow (29), Healy (30) and others. Fatalities have been attributed to thiocyanate therapy—the latest being described by Healy (30), Goldring and Chasis (31) and Garvin (32). Weakness and pain resembling angina pectoris were illustrated by Palmer and Sprague (33). Careful analysis and classification of the various untoward effects from the use of thiocyanate were made by Goldring and Chasis (31), and particularly by Wald, Lindberg and Barker (34).

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† From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis.